Trinidadian guppies use a social heuristic that can support cooperation among non-kin

Safi K. Darden[1*], Richard James[2], James M. Cave[2], Josefine Bohr Brask[1] and Darren P. Croft[1]

[1]Centre for Research in Animal Behaviour, Department of Psychology, College of Life and Environmental Sciences, University of Exeter, UK

[2]Department of Physics and Centre for Networks and Collective Behaviour, University of Bath, UK

Short title: Social heuristics and real-world cooperation

*Correspondence: s.darden@exeter.ac.uk

Abstract

Cooperation among non-kin is well documented in humans and widespread in non-human animals, but explaining the occurrence of cooperation in the absence of inclusive fitness benefits has proven a significant challenge. Current theoretical explanations converge on a single point: co-operators can prevail when they cluster in social space. However, we know very little about the real-world mechanisms that drive such clustering, particularly in systems where cognitive limitations make it unlikely that mechanisms such as score keeping and reputation are at play. Here we show that Trinidadian guppies (*Poecilia reticulata*) use a 'Walk Away' strategy, a simple social heuristic by which assortment by cooperativeness can come about among mobile agents. Guppies cooperate during predator inspection and we found that when experiencing defection in this context, individuals prefer to move to a new social environment, despite having no prior information about this new social group. Our results provide evidence in non-human animals that individuals use a simple social partner updating strategy in response to defection, supporting theoretical work applying heuristics to understanding the proximate mechanisms underpinning the evolution of cooperation among non-kin.

Introduction

The conundrum of cooperation [1, 2], where one individual pays a cost so that another can receive a benefit, was highlighted by Darwin [3], who realised that individuals that express a trait (e.g., cooperation) must themselves benefit for the trait to be favoured by natural selection. Yet cooperation is seen at every level of biological organization (intra cellular to societal) [4] and across taxonomic groups from microbes to humans [1]. Cooperation becomes particularly difficult to explain when benefits are conferred upon unrelated individuals and the past three decades have seen substantial theoretical attention given to identifying pathways by which non-kin cooperation can evolve (e.g. direct reciprocity [5], indirect reciprocity [6], generalised reciprocity [7-9], network reciprocity [10], group selection [11] and by-product benefits [12]). The merits of each of these models have been much debated [13-17], but they all have a single unifying feature: for cooperation to persist, co-operating individuals must cluster together [reviewed in 18]. Essentially, cooperation can prevail when cooperative individuals interact at higher rates with each other than with non-cooperative individuals, because this decreases the exploitation of cooperators by defectors and increases reciprocation of cooperative benefits to cooperators. Thus clusters of co-operators can gain higher fitness payoffs than defectors in the population [19, 20]. Identifying the processes that drive the clustering of cooperation in social landscapes is thus at the heart of unravelling the conundrum of how costly behaviours that benefit non-kin have evolved [19].

Theoretical work suggests that heuristics, simple decision-making rules, can underpin social dynamics (the formation and breaking of social ties) and thereby drive assortment by cooperation [18, 21-23]. For example, decisions about joining or leaving groups in response to cooperation or defection can generate social assortment by individual cooperativeness (i.e.

phenotypic propensity to cooperate) [18, 22-24]. Heuristics incorporate behavioural rules for making fast and economical decisions when the information available to individuals is incomplete and the future is uncertain [25]. These conditions for decision making are likely to be prevalent in systems with noisy, rapidly varying social environments and where decision making is not supported by advanced cognitive abilities; conditions which typify many non-human social animals. Currently however, it is unclear whether heuristics have a role to play in driving the dynamical linking of social ties in non-human animals in the context of cooperation. This represents a key gap in understanding cooperation, as characterizing the behavioural rules that govern dynamical linking is fundamental to determining the mechanisms that drive the clustering of co-operators [26]. Here we probe the social heuristics that underpin the formation and breaking of social ties in the context of cooperation in Trinidadian guppies (*Poecilia reticulata*).

Trinidadian guppies live in dynamic fission-fusion societies where individuals cooperate with non-kin during predator inspection [27] and where there is evidence of social assortment by cooperative tendency [28]. During predator inspection in fish, one or more individuals will leave the shoal to approach the predator closely and gain information about the level of threat posed by the predator [29]; information that benefits all members of the group [30]. Work in guppies and other fish species has demonstrated that inspectors pay a personal cost of increased risk of predation [31, 32], which they can reduce by inspecting in cooperative partnerships [33-35]. There has been much debate on the mechanisms maintaining cooperation during predator inspection, with some evidence suggesting a 'TIT-for-TAT' strategy is used [36]. In this strategy, individuals initially cooperate with a partner and in future, repeated iterations with this same partner, copy the partner's last move (i.e. either cooperate or defect) [36]. Given the highly dynamic nature of daily social interactions however, and the large number of individuals

that make up each individual's social environment [37, 38], guppies are also likely to rely on simple behavioural mechanisms of assortment that will allow them to avoid having to process and store the high volumes and rates of social information that they are exposed to. Guppies therefore constitute a potentially powerful model system for a new avenue of empirical work to test for key assortment mechanisms proposed by theoretical models to underpin the evolution of cooperation among unrelated individuals.

We aimed to test whether individuals use a simple behavioural strategy - 'leave in the face of defection' requiring only limited information on the behavioural tendencies of others. Models by Aktipis [18, 22] and Schuessler [24] show that such simple heuristics can generate assortment among cooperative mobile agents. Under a 'Walk Away' conditional movement strategy, individuals break away from defecting social partners [18, 22-24] and join a new partner or group upon encounter, without information on the behavioural tendencies of the partner or group [18, 22]. The conceptual attraction of the 'Walk Away' heuristic for generating positive assortment of cooperative phenotypes in real-world populations is that it avoids cognitively demanding bookkeeping. That is, it does not require committing to memory the identity of social partners, or indeed their behaviour over multiple iterations, to aid in making decisions to associate with a partner (or partners). This is in contrast to the TIT-for-TAT strategy, which requires remembering the last actions of specific partners (i.e. partner behaviour and identity). The strategy also differs from other exit strategies such as the well-known 'win-stay, lose-shift', where an actor continues or "stays" with an action – cooperate or defect - unless the gain no longer meets some threshold and then switches or "shifts" to the opposing action - cooperate or defect in an iterated game [39]. Like with a 'TIT-for-TAT' strategy, an individual thus changes their own cooperativeness as a reaction to that of others [although for an approach that models 'win-stay, lose-shift' with 'shift' including an option to

leave the group see 23]. In contrast, in the 'Walk Away' strategy individuals in effect change their social environment without any prescription for who to join or how to behave (cooperate or defect) in any subsequent round or game [18, 22, 24]. That is, with a 'Walk Away' strategy, individuals do not need to be able to exhibit plasticity in their own cooperative behaviour, further contributing to its simplicity and, importantly, possible traits under selection [e.g., 40, 41-43].

'Walk Away' models for the evolution of cooperation were originally formulated for populations with fairly stable group structures [18, 22, 24]. However, populations of social animals typically live in societies with fission-fusion dynamics, such as those experienced by Trinidadian guppies. It is not immediately clear that under these conditions, a 'Walk Away' strategy can allow positive assortment of cooperation to emerge against the background merging and splitting of groups, which in this and other systems is driven by myriad factors [44]. We have therefore confirmed that a 'Walk Away' social heuristic can generate assortment by cooperation in populations with fission-fusion dynamics similar to those in guppies using an agent-based simulation model to further support the rationale for the current study (see supplementary materials). To test the hypothesis that guppies will use a 'Walk Away' strategy, we exposed individuals to unfamiliar social partners, manipulated their perception of these partners' cooperative behaviour during a predator inspection event and then monitored the propensity for individuals to change their social environment following their ostensible experience of cooperation or defection. We predicted that if a 'Walk Away' strategy exists in this species, individuals would prefer to associate with novel social partners over social partners that they had just experienced defection from.

Methods

131 ***Study animals***

132 We used laboratory reared adult female Trinidadian guppies descended from wild fish collected
133 in the lower reaches of the Aripo River (10°40' N 61° 14' W) on the island of Trinidad, a site
134 where adult guppies experience a high risk of predation from piscivorous fish. Focal fish were
135 housed in groups of 10 in 29 x 19 x 17 cm aquaria. Stimulus fish were housed in groups of 100
136 in 80 x 30 x 39 cm aquaria. Focal and stimulus fish were randomly selected from stocks of fish
137 housed under naturalistic conditions in four physically isolated pools (approximately 2000 fish
138 per pool). All fish were fed twice daily to satiation on their specified diet (stimulus fish diets
139 are explained below; focal fish were fed on a diet of tropical fish flake and brine shrimp,
140 *Artemia* sp.). The study was carried out under UK Home Office Licence PIL 30/8944, reviewed
141 by the University of Exeter Animal Welfare and Ethical Review Body, and in strict accordance
142 with the UK Animals (Scientific Procedures) Act 1986. To minimize stress, all fish used in the
143 study were provided with plant refugia and always had, at a minimum, visual access to social
144 partners, with the exception of our control experiment where focal fish were without contact to
145 social partners during testing. Power analysis after an initial data collection phase (N=6
146 replicates per cell) was used to ensure that we used the smallest number of animals possible
147 while maintaining high test power (16 replicates per cell, SPSS SamplePower 21 v. 3.0.1, IBM
148 SPSS Inc.).
149
150 ***Experimental apparatus and procedure***
151
152 *Study design*
153
154 To test for the existence of a 'Walk Away' strategy in Trinidadian guppies, we experimentally
155 exposed 136 female guppies to a cooperative or non-cooperative social environment and

subsequently tested their social preference for ostensibly the same social environment versus a novel one.

Predator inspection

Inspection arenas were similar to those used in other studies involving predator inspection in guppies (e.g., [28, 45, 46]). Aquaria (80 x 30 cm) were sub-divided with Perspex partitions to produce two inspection lanes and two predator enclosures (Fig. 1A). A guide system was in place between the predator enclosures and the inspection lanes where a removable opaque partition was positioned to visually isolate the predator enclosure from focal fish prior to the start of a trial. Predator enclosures were either empty or contained a single predatory fish (*Aequidens pulcher*) depending on condition (see below). A refuge was located at the end opposite to the predator enclosures with an artificial plant and a perforated transparent rectangular stimulus shoal compartment (10 x 4.5 x 18 cm). The inside of each inspection lane was lined with a reversible partition that had a mirror on one side and a uniform, light grey surface on the other side. With this design, in a mirrored lane an inspecting fish was ostensibly joined by a fish from the compartment of social partners (i.e. the stimulus shoal) in the form of its mirrored reflection, and in a non-mirrored lane also connected to a compartment of physically constrained social partners an inspecting fish ostensibly experienced defection from these partners (Fig. 1A). This experimental paradigm built on protocols used in previous studies [reviewed in 47], and recent work has illustrated that using a mirror stimulus in a predator inspection context elicits behaviour in a focal fish that aligns with its behaviour with a live partner [28]. The water depth in each subsection of an arena was 11 cm. Arenas were illuminated with full spectrum 40W bulbs and filmed from above using Samsung digital colour cameras (Model: SCB-2001) fitted with a Computar 5-50mm, F1:1.3 lens.

Thirty minutes before the onset of each trial, predator naïve stimulus shoals were placed in the stimulus shoal compartments of each inspection lane. Each stimulus shoal consisted of four size-matched, predator-naïve female guppies that the focal fish had not previously encountered. We manipulated identity cues of the stimulus shoals by feeding them on one of two diets (larval *Chironominae* sp. or adult *Daphnia* sp.) that were novel to the focal fish, for min. 7 days and up to 14 days prior to the trials. Guppies use odour cues for social decision making [48] and this method allowed us to generate distinct novel odour cues for groups of fish. Stimulus shoal compartment walls were perforated to allow odour cues to diffuse across the compartment barrier. During their inspection of the predator (Fig. 1A) focal fish could thus become familiar with global (shoal level) odour cues of social partners originating from their diet in tandem with experiencing either defection or cooperation, depending on treatment.

At the start of a trial, individual focal fish were released into the centre of an inspection lane and allowed 10 minutes to acclimatize. During this period the opaque partition between the predator enclosures and the inspection lanes remained in place. Focal individuals were then gently encouraged into the refuge area next to the confined stimulus shoal using a dip net. The opaque partition between the predator enclosures and inspection lanes was then lifted. In experimental test trials the lifting of the barrier revealed a live predator and in control trials, intended to account for possible effects inherent to the experimental setup, an empty enclosure. Inspection occurred when fish left the refuge area and swam towards the predator enclosure. Mirrored lanes simulated cooperation by a member of the stimulus shoal, while non-mirrored lanes simulated defection by all members of the shoal. Trials ended after a 5-minute inspection period and focal fish were immediately removed from the inspection lane and transferred in a small container of water into a binary choice tank for the social partner choice test (see below).

206 At the end of a trial all stimulus fish were removed and a complete water change of the arena
207 was carried out.
208
209 *Social partner choice test*
210
211 Immediately following the predator inspection trial, focal individuals were transferred to a
212 binary shoal choice arena and tested for their association preferences for social partners fed
213 either on the same diet as experienced in the predator inspection trial (i.e. *Chironominae* sp. or
214 *Daphnia* sp. fed fish) or the unfamiliar (novel) diet. Arenas (45 x 30 cm, water depth 11cm)
215 were sub-divided into three compartments using perforated Perspex barriers similar to [49].
216 Two stimulus shoal compartments at opposite ends of the arena measured 7.5 x 30 cm, which
217 left a middle compartment for the focal fish that measured 30 x 30 cm. Arenas were illuminated
218 and filmed as above. Forty-five minutes prior to the onset of a trial a shoal of 5 fish was placed
219 in each stimulus shoal compartment of the choice arena (matched for body size across shoals).
220 One compartment contained fish on the *Daphnia* sp. diet and the other contained fish on the
221 bloodworm diet. Each focal fish was thus presented with one stimulus shoal composed of fish
222 on the same diet as the fish they had experienced in the inspection trial and another composed
223 of fish on the second novel diet, to which the focal was naive. All stimulus fish were predator
224 naïve and had not been used in the predator exposure treatment. This design was used because
225 the experiences of the stimulus fish during the inspection trials could potentially lead to
226 differential behaviour between the two shoals during the choice trial if they were used there as
227 well. Using odours as identity cues allowed us to avoid this potentially confounding factor. At
228 the start of a choice trial, focal fish that had just been removed from an inspection trial were
229 placed in the centre of the arena and given 5 minutes to acclimatize. After acclimatization, we
230 recorded the time that focals spent shoaling with each stimulus shoal over a 10-minute period.

Focal fish were recorded as shoaling with stimulus fish if they were within 5 cm of the barrier to a shoal compartment (preference zone; based on the elective group size concept [50]). At the end of the trial all fish were removed from the arena and a complete water change was carried out.

Analysis of behavioural data

Our analysis is based on 129 focal fish that entered the preference zone of both shoals at least once during the shoal choice trial (7 fish did not visit both sides; Supplementary material Table S1). The inspection and shoaling behaviour of each focal fish was scored manually using the Observer XT v. 10.1 by a single observer (SKD) blind to the condition and treatment that focal fish were in. For inspection trials we quantified the average distance of focal fish to the predator enclosure over the 5-minute inspection period. For shoal choice trials we calculated the proportion of shoaling time that focal fish spent with each of the two shoals which were angular transformed prior to statistical analysis as per convention for analysing proportional data in this way [51].

We used a general linear model (GLM) to test for effects of our experimental manipulations on the social partner choices made by our focal fish. In the model we used the angular transformed proportion of time spent with the novel (unfamiliar odour) shoal during the binary shoal choice trial as the dependent variable, and condition (2 levels: control and experimental), social experience (2 levels: defection and cooperation) and stimulus shoal diet encountered during inspection (2 levels: *Daphnia* and bloodworm) as fixed effects..Our initial model contained the inspection behaviour of our focal fish as a covariate, however it had no effect ($F_{1,116}=0.393$; $p=0.532$, see Supplementary materials Table S2)) and was removed from the

final model. We explored a significant interaction between condition and treatment using *post hoc* one-sample t-tests with a Bonferroni corrected α level of 0.0125.

Methods of non-social control experiment

We ran a non-social control experiment that used a modified version of the main experimental paradigm in order to investigate whether any effects found in the main experiment could alternatively be explained by the guppies connecting their experience (cooperation/defection) with the odour cues themselves, rather than with the social environments associated with those odour cues. That is, effects found in the main experiment could potentially be explained by a mechanism that caused focal individuals to, for example, avoid an odour that they associated with high predation risk in the defection condition (approaching a predator as a singleton). In this control experiment, the overall design was the same as in the main experiment (inspection then shoal choice) and odour cues derived from the same diets were used (*Chironominae* sp. and *Daphnia* sp.; see below)), but no social cues (no stimulus shoal and no mirror) were provided in the inspection trials. In the subsequent shoal choice test, focal individuals could choose between two shoals of fish, each of which was paired with one of the two odours.

Odour cues in this experiment were introduced in the form of odour water. This was created by masticating frozen daphnia or bloodworm (*Daphnia* sp., and *Chironominae* sp., i.e. the same diet odours as in the main experiment) in water (5 g of daphnia and 2.6 g of bloodworm per 300 ml water) and filtering the mixture through a fine sieve in order to remove macroscopic particles. The odour water was introduced into the predator inspection lane at the refuge end, where the stimulus shoal was placed in the main experiment (opposite to the predator stimulus end), via a plastic tube connected to a funnel placed over the tank. The rate at which the odour

281 water entered the tank was controlled by a flowmeter (MMA-35, Dwyer Instruments, Michigan
282 City, IN, USA) set to 25 ml/min. 500ml odour water was placed in the funnel prior to the trial
283 and the flowmeter was opened at the beginning of the trial. The trial otherwise proceeded as in
284 the main experiment (as per above in a 'no mirror' condition only). The subsequent binary
285 shoal choice tests were also similar to the ones in the main experiment; except that the stimulus
286 shoals each consisted of four females that had not been fed with the diets used to create odours.
287 Instead, odour water (200 ml) with the two experimental odours was introduced into each shoal
288 compartment prior to the test trial, one odour in each compartment. The experimental tanks
289 were thoroughly cleaned after each trial to remove any odour remains. We used a one-sample
290 t-test to test for a preference for shoals paired with the novel odour, taken as the angular
291 transformation of the proportion of shoaling time spent with this shoal.
292
293 **Results**
294
295 We found that the presence or absence of a predator during the inspection portion of a trial (i.e.
296 inspection condition: experimental or control) interacted with having partners that either
297 cooperated or defected during the inspection (i.e. social experience: cooperation or defection)
298 to influence subsequent shoal choice (Table 1). *Post hoc* analysis revealed that individuals
299 experiencing a defecting social environment preferred partners with an unfamiliar odour over
300 partners with a familiar odour when given a subsequent choice (Fig. 1B), which was not the
301 case for control treatments (no predator) or our experimental cooperation treatment, where we
302 did not find any preferences (Table 2, Bonferroni corrected α=0.0125).
303
304 If the significant preference found in the main experiment was based on avoidance of the odour
305 associated with inspecting a predator as a singleton, rather than avoidance of the social

environment associated with the predator inspection experience, then a preference for a shoal bearing a novel odour (as opposed to that experienced during inspection), should also be present in the non-social control experiment. However, in this control experiment we found that focal fish did not show a preference for fish associated with the novel odour (back-transformed mean proportion of time spent with novel odour fish+/-SE=0.448+0.0406/-0.0403; t_{62}=-1.275, p=0.2070).

Discussion

We found that female Trinidadian guppies experiencing a social environment where all others defected during predator inspection, preferred novel partners (that they had no prior information on) over ostensibly familiar social partners in a subsequent social choice test. This result demonstrates that individuals actively sever ties with defecting social partners and seek out links with others, even when they do not have information on the cooperative behaviour of these novel social partners; both are consistent with a 'Walk Away' strategy [18, 22, 24]. To our knowledge this is the first empirical evidence for the existence of this social heuristic in a non-human animal system.

In humans the option to leave a defecting partner, 'opting out', has been shown both theoretically and empirically to allow cooperation to prevail [52-56] and empirical work suggests that something akin to conditional movement strategies is active in humans. For example, dynamic partner updating under conditions of limited information has been demonstrated experimentally in response to low levels of cooperative behaviour in partners [40, 52, 54, 55, 57]. Indeed, one study has shown that when constrained to a set behavioural repertoire of either staying with an interaction partner or joining another, randomly assigned,

partner between rounds of a cooperative game, movement ('link-breaking') decisions generate assortment of cooperative behaviour across a network of interaction partners [40]. It is important to note that in the majority of paradigms in these empirical studies with humans, participants operate with partner-specific information that goes beyond what is outlined for a 'Walk Away' strategy, so that ties are preferentially broken with defectors and new ties are preferentially made with co-operators [e.g., 52, 57] or individuals are able to log the behaviour of specific individuals and use this knowledge in subsequent encounters with those individuals [40]. Still, at the core of these paradigms, having knowledge of and control over the option to leave is critical in determining the economic decisions made by players [52, 54, 55, 58, 59], even when the assignment of a new partner is made at random [54, 55, 59]. Our study provides evidence of the existence of this class of strategies outside of humans and supports its simplest use, with individuals making social association choices when they have no information on the value of future partners. The simplicity of this strategy means that it may be widespread in natural systems [60]. Furthermore, future work examining the heritability of the 'Walk Away' strategy and how it has been shaped by natural selection would provide valuable insights into the evolution of cooperation in natural populations.

Although our findings highlight a mechanism thay may go some way to explaining the persistence of non-kin cooperation in guppy populations, they do not preclude other mechanisms that may be working simultaneously in this species; such as choosing specific partners based on immediate observation of their cooperative tendency [e.g., 61, but see below] or conditional cooperative behaviour based on the cooperative behaviour of current social partners [10, 62-64]. For example, generalized reciprocity (or 'help anyone if helped by someone'), has been demonstrated with computer modelling to generate positive assortment of cooperative interactions via cooperative responses conditional to experience [65]. Support for

356 cooperation via generalized reciprocity is based on experiences of cooperative behaviour that
357 is wholly anonymous (i.e. identification of the actor is not necessary), and thus may be
358 particularly relevant for the guppy system [23,65-66]. Future work exploring if other social
359 heuristics are used in combination with a 'Walk Away' strategy to support cooperation in
360 guppies is eagerly anticipated.
361
362 In our experiment, in addition to guppies 'walking away' from defecting partners it could also
363 be expected that they would prefer the social environment where they had experienced
364 cooperation. Both of these would work toward driving the positive assortment by cooperative
365 propensity [reviewed in 67 and see Supplementary materials Section 1] that we have seen
366 evidence for in wild guppy populations [28]. We did not, however, find clear evidence that our
367 focal individuals preferred partners that had cooperated during predator inspection over
368 partners for whom they had no information on their propensity to cooperate. Previous evidence
369 from this study system indeed suggests that individuals have a preference for a more
370 cooperative over a less cooperative partner when given a choice between the two [61]. .
371 However, a key paradigm difference between the experiment presented here and this previous
372 work [61] is that individuals were able to choose from social partners for whom they had
373 complete information; that is, they had knowledge of the cooperative propensity of each
374 potential partner in a binary choice test. This means that although fish may have been actively
375 choosing the more cooperative partner, they may alternatively have been actively choosing to
376 leave the defecting partner as in our study. In support of this latter explanation, we can consider
377 evidence from work in humans suggesting a higher propensity to remember traits or
378 experiences associated with defectors compared to cooperators [68]. In humans this effect
379 appears to be linked to the importance of the information in predicting trait characteristics of
380 individuals and thus the outcome of future interactions [69, 70]. In this case, a negativity bias

can exist when 'negative' cues are more diagnostic than 'positive' cues [70]. With a 'Walk Away' heuristic, the important diagnostic information regarding the behaviour of an unfamiliar social group is whether they defect during predator inspection, as opposed to whether they cooperate, as this is what drives the decision to leave. It could be that the underlying premise for this strategy is a negativity bias, particularly when an entire group of individuals defects compared to when just one individual from a group cooperates (i.e. the diagnostic value of the 'positive' information is low). An increased propensity to remember social partners from a situation where they defected, but not where they cooperated, and then acting on this information for subsequent social association decisions, thus seem like plausible explanations for the updating behaviour and lack of preference for cooperative shoals that we observed.

Theoretical work over the last decade has striven to identify simple behavioural mechanisms that can maintain cooperation among non-kin [most recently reviewed in 63, 67, 71], with social heuristics likely being important drivers in systems with high levels of social mixing [e.g., 72]. In our experimental design, individuals did not have the opportunity to use individual recognition or other information when making partner choices. The work we present thus truly represents evidence of a real-world heuristic for dynamical linking of social ties in non-human animals. It most closely resembles a 'Walk Away' heuristic, which can generate positive social assortment by cooperative behaviour in populations of mobile agents ([18] and see Supplementary materials Section 1). The simplicity of this strategy means that it may be a general mechanism contributing to the maintenance of cooperation across a broad range of taxa where individuals can detect non-cooperative behaviour, but where more complex processes involving, for example, intent and knowledge attribution or bookkeeping of behaviour [73-76], are not necessarily present. We look forward to further developments in this area.

406 **Acknowledgements**

407

408 We thank R. Cope and M. Edenbrow for assistance with data collection and M. Cant and our
409 reviewers for comments on earlier versions of the manuscript.

410

411 **Funding**

412

413 The study was funded by a Leverhulme Trust Early Career Fellowship (ECF/2010/0672) to
414 SKD, a Leverhulme Trust Research Grant (RPG-175) to DPC and a Framework Grant (DFF –
415 1323-00105) to SKD and DPC from the Danish Research Council for Nature and Universe.
416 JBB was supported by an internationalisation postdoc fellowship from the Carlsberg
417 Foundation.

418

419 **Ethics**

420

421 The study was carried out under UK Home Office Licence PIL 30/8944, reviewed by the
422 University of Exeter Animal Welfare and Ethical Review Body, and in strict accordance with
423 the UK Animals (Scientific Procedures) Act 1986.

424

425 **Data, code and materials**

426

427 Data: http://hdl.handle.net/10871/18463

428

429 **Competing interests**

430

The authors declare no competing interests.

Authors' contributions

The main empirical study was conceived and designed by SKD and DPC and data collection overseen by SKD. The empirical validation study was designed and carried out by JBB in discussion with SKD. The simulation model was conceptualized by SKD, RJ and DPC, designed by RJ and implemented by JC. SKD wrote the first draft of the manuscript in discussion with DPC. All authors contributed to subsequent revisions. SKD and DPC designed and produced the figures in discussion with RJ.

References

[1] Pennisi, E. 2009 On the Origin of Cooperation. *Science* **325**, 1196.

[2] Hamilton, W.D. 1963 Evolution of Altruistic Behavior. *American Naturalist* **97**, 354-356.

[3] Darwin, C. 1859 *The Origin of Species*. London, J. Murray.

[4] Queller, D.C. & Strassmann, J.E. 2009 Beyond Society: The Evolution of Organismality. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**, 3143-3155.

[5] Trivers, R.L. 1971 Evolution of Reciprocal Altruism. *Quarterly Review of Biology* **46**, 35-57.

[6] Nowak, M.A. & Sigmund, K. 1998 Evolution of Indirect Reciprocity by Image Scoring. *Nature* **393**, 573-577. (doi:10.1038/31225).

[7] Pfeiffer, T., Rutte, C., Killingback, T., Taborsky, M. & Bonhoeffer, S. 2005 Evolution of Cooperation by Generalized Reciprocity. *Proceedings of the Royal Society B: Biological Sciences* **272**, 1115-1120.

[8] Nowak, M.A. & Roch, S. 2007 Upstream Reciprocity and the Evolution of Gratitude. *Proceedings of the Royal Society B: Biological Sciences* **274**, 605-610.

[9] Rutte, C. & Taborsky, M. 2007 Generalized Reciprocity in Rats. *Plos Biology* **5**, 1421-1425. (doi:10.1371/journal.pbio.0050196).

[10] Ohtsuki, H. & Nowak, M.A. 2007 Direct Reciprocity on Graphs. *Journal of Theoretical Biology* **247**, 462-470. (https://doi.org/10.1016/j.jtbi.2007.03.018).

[11] Wilson, D.S. 1975 Theory of Group Selection. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 143-146.

[12] Brown, J.L. 1983 Cooperation—a Biologist's Dilemma. In *Advances in the Study of Behavior* (eds. J.S. Rosenblatt, R.A. Hinde, C. Beer & M.-C. Busnel), pp. 1-37, Academic Press.

[13] Wilson, D.S. 2008 Social Semantics: Toward a Genuine Pluralism in the Study of Social Behaviour. *Journal of Evolutionary Biology* **21**, 368-373. (doi:10.1111/j.1420-9101.2007.01396.x).

[14] Nowak, M.A. 2006 Five Rules for the Evolution of Cooperation. *Science* **314**, 1560-1563. (doi:10.1126/science.1133755).

[15] Wilson, E.O. 2005 Kin Selection as the Key to Altruism: Its Rise and Fall. *Social Research* **72**, 159-166.

[16] West, S.A., Griffin, A.S. & Gardner, A. 2007 Social Semantics: Altruism, Cooperation, Mutualism, Strong Reciprocity and Group Selection. *Journal of Evolutionary Biology* **20**, 415-432. (doi:10.1111/j.1420-9101.2006.01258.x).

[17] Foster, K.R., Wenseleers, T. & Ratnieks, F.L.W. 2006 Kin Selection Is the Key to Altruism. *Trends in Ecology & Evolution* **21**, 57-60. (https://doi.org/10.1016/j.tree.2005.11.020).

[18] Aktipis, C.A. 2011 Is Cooperation Viable in Mobile Organisms? Simple Walk Away Rule Favors the Evolution of Cooperation in Groups. *Evolution and Human Behavior* **32**, 263-276. (http://dx.doi.org/10.1016/j.evolhumbehav.2011.01.002).

[19] Fletcher, J.A. & Doebeli, M. 2009 A Simple and General Explanation for the Evolution of Altruism. *Proceedings of the Royal Society B: Biological Sciences* **276**, 13-19.

[20] Nowak, M.A., Tarnita, C.E. & Antal, T. 2010 Evolutionary Dynamics in Structured Populations. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 19-30.

[21] Pepper, J.W. & Smuts, B.B. 2002 A Mechanism for the Evolution of Altruism among Nonkin: Positive Assortment through Environmental Feedback. *American Naturalist* **160**, 205-213.

[22] Aktipis, C.A. 2004 Know When to Walk Away: Contingent Movement and the Evolution of Cooperation. *Journal of Theoretical Biology* **231**, 249-260.

[23] Hamilton, I.M. & Taborsky, M. 2005 Contingent Movement and Cooperation Evolve under Generalized Reciprocity. *Proceedings of the Royal Society B: Biological Sciences* **272**, 2259-2267. (doi:10.1098/rspb.2005.3248).

[24] Schuessler, R. 1989 Exit Threats and Cooperation under Anonymity. *The Journal of Conflict Resolution* **33**, 728-749.

[25] Gigerenzer, G. & Gaissmaier, W. 2011 Heuristic Decision Making. *Annual Review of Psychology* **62**, 451-482. (doi:10.1146/annurev-psych-120709-145346).

501 [26] Pacheco, J.M., Traulsen, A. & Nowak, M.A. 2006 Co-Evolution of Strategy and Structure
502 in Complex Networks with Dynamical Linking. *Physical review letters* **97**, 258103-
503 258103.
504 [27] Croft, D., Hamilton, P.B., Darden, S.K., Jacoby, D.M.P., James, R., Bettaney, E.M. &
505 Tyler, C.R. 2012 The Role of Relatedness in Structuring the Social Network of a Wild
506 Guppy Population. *Oecologia* **170**, 955-963. (doi:10.1007/s00442-012-2379-8).
507 [28] Brask, J.B., Croft, D.P., Edenbrow, M., James, R., Bleakley, B.H., Ramnarine, I.W.,
508 Heathcote, R.J.P., Tyler, C.R., Hamilton, P.B., Dabelsteen, T., et al. 2019 Evolution of
509 Non-Kin Cooperation: Social Assortment by Cooperative Phenotype in Guppies. *Royal*
510 *Society Open Science* **6**, 181493. (doi:10.1098/rsos.181493).
511 [29] Pitcher, T.J., Green, D.A. & Magurran, A.E. 1986 Dicing with Death - Predator Inspection
512 Behaviour in Minnow Shoals. *Journal of Fish Biology* **28**, 439-448.
513 [30] Magurran, A.E. & Higham, A. 1988 Information Transfer across Fish Shoals under
514 Predator Threat. *Ethology* **78**, 153-158.
515 [31] Dugatkin, L.A. 1992 Tendency to Inspect Predators Predicts Mortality Risk in the Guppy
516 (*Poecilia Reticulata*). *Behavioral Ecology* **3**, 124-127.
517 [32] Milinski, M., Luthi, J.H., Eggler, R. & Parker, G.A. 1997 Cooperation under Predation
518 Risk: Experiments on Costs and Benefits. *Proceedings of the Royal Society B: Biological*
519 *Sciences* **264**, 831-837.
520 [33] Dugatkin, L.A. 1988 Do Guppies Play Tit for Tat During Predator Inspection Visits.
521 *Behavioural Ecology and Sociobiology* **23**, 395-399.
522 [34] Milinski, M. 1987 Tit-for-Tat in Sticklebacks and the Evolution of Cooperation. *Nature*
523 **325**, 433-435.

524 [35] Croft, D.P., James, R., Thomas, P.O.R., Hathaway, C., Mawdsley, D., Laland, K.N. &
525 Krause, J. 2006 Social Structure and Co-Operative Interactions in a Wild Population of
526 Guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* **59**, 644-650.
527 [36] Dugatkin, L.A. & Alfieri, M. 1991 Tit-for-Tat in Guppies (*Poecilia reticulata*) - the
528 Relative Nature of Cooperation and Defection During Predator Inspection. *Evolutionary*
529 *Ecology* **5**, 300-309.
530 [37] Croft, D.P., Arrowsmith, B.J., Bielby, J., Skinner, K., White, E., Couzin, I.D., Magurran,
531 A.E., Ramnarine, I. & Krause, J. 2003 Mechanisms Underlying Shoal Composition in the
532 Trinidadian Guppy (*Poecilia reticulata*). *Oikos* **100**, 429-438.
533 [38] Croft, D.P., Krause, J. & James, R. 2004 Social Networks in the Guppy (*Poecilia*
534 *reticulata*). *Proceedings of the Royal Society B: Biological Sciences* **271**, S516-S519.
535 [39] Nowak, M. & Sigmund, K. 1993 A Strategy of Win-Stay, Lose-Shift That Outperforms
536 Tit-for-Tat in the Prisoner's Dilemma Game. *Nature* **364**, 56-58. (doi:10.1038/364056a0).
537 [40] Fehl, K., van der Post, D.J. & Semmann, D. 2011 Co-Evolution of Behaviour and Social
538 Network Structure Promotes Human Cooperation. *Ecology Letters* **14**, 546-551.
539 (doi:10.1111/j.1461-0248.2011.01615.x).
540 [41] dos Santos, M. & West, S.A. 2018 The Coevolution of Cooperation and Cognition in
541 Humans. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20180723.
542 (doi:10.1098/rspb.2018.0723).
543 [42] Geoffroy, F., Baumard, N. & André, J.-B. 2019 Why Cooperation Is Not Running Away.
544 *Journal of Evolutionary Biology* **32**, 1069-1081. (doi:10.1111/jeb.13508).
545 [43] Smead, R. & Forber, P. 2016 The Coevolution of Recognition and Social Behavior.
546 *Scientific Reports* **6**, 25813. (doi:10.1038/srep25813).
547 [44] Krause, J. & Ruxton, G.D. 2002 *Living in Groups*. Oxford, Oxford University Press.

548 [45] Dimitriadou, S., Croft, D.P. & Darden, S.K. 2019 Divergence in Social Traits in
549 Trinidadian Guppies Selectively Bred for High and Low Leadership in a Cooperative
550 Context. *Scientific Reports* **9**, 17194. (doi:10.1038/s41598-019-53748-4).
551 [46] Edenbrow, M., Bleakley, B.H., Darden, S.K., Tyler, C.R., Ramnarine, I.W. & Croft, D.P.
552 2017 The Evolution of Cooperation: Interacting Phenotypes among Social Partners. *The*
553 *American Naturalist* **189**, 630-643. (doi:10.1086/691386).
554 [47] Soares, M.C., Cardoso, S.C., Carvalho, T.d.S. & Maximino, C. 2018 Using Model Fish to
555 Study the Biological Mechanisms of Cooperative Behaviour: A Future for Translational
556 Research Concerning Social Anxiety Disorders? *Progress in Neuro-Psychopharmacology*
557 *and Biological Psychiatry* **82**, 205-215. (https://doi.org/10.1016/j.pnpbp.2017.11.014).
558 [48] Ward, A.J.W., Webster, M.M., Magurran, A.E., Currie, S. & Krause, J. 2009 Species and
559 Population Differences in Social Recognition between Fishes: A Role for Ecology?
560 *Behavioral Ecology* **20**, 511-516. (doi:10.1093/beheco/arp025).
561 [49] Darden, S.K., James, R., Ramnarine, I.W. & Croft, D.P. 2009 Social Implications of the
562 Battle of the Sexes: Sexual Harassment Disrupts Female Sociality and Social Recognition.
563 *Proceedings of the Royal Society B: Biological Sciences* **276**, 2651-2656.
564 (doi:10.1098/rspb.2009.0087).
565 [50] Pitcher, T.J., Magurran, A.E. & Allan, J.R. 1983 Shifts of Behaviour with Shoal Size in
566 Cyprinids. In *Proceedings of the 3rd British Freshwater Fish Conference* (pp. 220-228).
567 [51] Sokal, R.R. & Rohlf, F.J. 1995 *Biometry: The Principles and Practice of Statistics in*
568 *Biological Research*. New York, W.H. Freeman and Co.
569 [52] Rand, D.G., Arbesman, S. & Christakis, N.A. 2011 Dynamic Social Networks Promote
570 Cooperation in Experiments with Humans. *Proceedings of the National Academy of*
571 *Sciences of the United States of America* **108**, 19193-19198.
572 (doi:10.1073/pnas.1108243108).

573 [53] Izquierdo, S.S., Izquierdo, L.R. & Vega-Redondo, F. 2010 The Option to Leave:
574 Conditional Dissociation in the Evolution of Cooperation. *Journal of Theoretical Biology*
575 **267**, 76-84. (doi:https://doi.org/10.1016/j.jtbi.2010.07.039).
576 [54] Barclay, P. & Raihani, N. 2016 Partner Choice Versus Punishment in Human Prisoner's
577 Dilemmas. *Evolution and Human Behaviour* **37**, 263-271.
578 (https://doi.org/10.1016/j.evolhumbehav.2015.12.004).
579 [55] Zhang, B.-Y., Fan, S.-J., Li, C., Zheng, X.-D., Bao, J.-Z., Cressman, R. & Tao, Y. 2016
580 Opting out against Defection Leads to Stable Coexistence with Cooperation. *Scientific*
581 *Reports* **6**, 35902. (doi:10.1038/srep35902
582 https://www.nature.com/articles/srep35902#supplementary-information).
583 [56] Zheng, X.-D., Li, C., Yu, J.-R., Wang, S.-C., Fan, S.-J., Zhang, B.-Y. & Tao, Y. 2017 A
584 Simple Rule of Direct Reciprocity Leads to the Stable Coexistence of Cooperation and
585 Defection in the Prisoner's Dilemma Game. *Journal of Theoretical Biology* **420**, 12-17.
586 (https://doi.org/10.1016/j.jtbi.2017.02.036).
587 [57] Wang, J., Suri, S. & Watts, D.J. 2012 Cooperation and Assortativity with Dynamic Partner
588 Updating. *Proceedings of the National Academy of Sciences of the United States of*
589 *America* **109,** 14363-14368. (doi:10.1073/pnas.1120867109).
590 [58] Boone, R.T. & Macy, M.W. 1999 Unlocking the Doors of the Prisoner's Dilemma:
591 Dependence, Selectivity, and Cooperation. *Social Psychology Quarterly* **62**, 32-52.
592 (doi:10.2307/2695824).
593 [59] Bednarik, P., Fehl, K. & Semmann, D. 2014 Costs for Switching Partners Reduce Network
594 Dynamics but Not Cooperative Behaviour. *Proceedings of the Royal Society B: Biological*
595 *Sciences* **281**, 20141661.

596 [60] Tomassini, M. & Antonioni, A. 2015 Lévy Flights and Cooperation among Mobile
597 Individuals. *Journal of Theoretical Biology* **364**, 154-161.
598 (doi:http://dx.doi.org/10.1016/j.jtbi.2014.09.013).
599 [61] Dugatkin, L.A. & Alfieri, M. 1991 Guppies and the Tit-for-Tat Strategy - Preference
600 Based on Past Interaction. *Behavioral Ecology and Sociobiology* **28**, 243-246.
601 [62] Smith, K.M., Larroucau, T., Mabulla, I.A. & Apicella, C.L. 2018 Hunter-Gatherers
602 Maintain Assortativity in Cooperation Despite High Levels of Residential Change and
603 Mixing. *Current Biology* **28**, 3152-3157. (doi:https://doi.org/10.1016/j.cub.2018.07.064).
604 [63] Quiñones, A.E., van Doorn, G.S., Pen, I., Weissing, F.J. & Taborsky, M. 2016 Negotiation
605 and Appeasement Can Be More Effective Drivers of Sociality Than Kin Selection.
606 *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**.
607 [64] Bshary, R. & Grutter, A.S. 2002 Asymmetric Cheating Opportunities and Partner Control
608 in a Cleaner Fish Mutualism. *Animal Behaviour* **63**, 547-555.
609 (https://doi.org/10.1006/anbe.2001.1937).
610 [65] Stojkoski, V., Utkovski, Z., Basnarkov, L. & Kocarev, L. 2018 Cooperation Dynamics of
611 Generalized Reciprocity in State-Based Social Dilemmas. *Physical Review E* **97**, 052305.
612 (doi:10.1103/PhysRevE.97.052305).
613 [66] Utkovski, Z., Stojkoski, V., Basnarkov, L. & Kocarev, L. 2017 Promoting Cooperation by
614 Preventing Exploitation: The Role of Network Structure. *Physical Review E* **96**, 022315.
615 (doi:10.1103/PhysRevE.96.022315).
616 [67] Croft, D.P., Edenbrow, M. & Darden, S. 2014 Assortment in Social Networks and the
617 Evolution of Cooperation. In *Animal Social Networks* (eds. J. Krause, R. James, D.W.
618 Franks & D.P. Croft), pp. 13-23, Oxford University Press, USA.

[68] Buchner, A., Bell, R., Mehl, B. & Musch, J. 2009 No Enhanced Recognition Memory, but Better Source Memory for Faces of Cheaters. *Evolution and Human Behaviour*. **30**, 212-224. (https://doi.org/10.1016/j.evolhumbehav.2009.01.004).

[69] Bell, R., Sasse, J., Möller, M., Czernochowski, D., Mayr, S. & Buchner, A. 2016 Event-Related Potentials in Response to Cheating and Cooperation in a Social Dilemma Game. *Psychophysiology* **53**, 216-228. (doi:10.1111/psyp.12561).

[70] Skowronski, J.J. & Carlston, D.E. 1987 Social Judgment and Social Memory - the Role of Cue Diagnosticity in Negativity, Positivity, and Extremity Biases. *Journal of Personality and Social Psychology* **52**, 689-699. (doi:10.1037/0022-3514.52.4.689).

[71] van Doorn, G.S. & Taborsky, M. 2011 The Evolution of Generalized Reciprocity on Social Interaction Networks. *Evolution* **66**, 651-664. (doi:10.1111/j.1558-5646.2011.01479.x).

[72] van den Berg, P. & Wenseleers, T. 2018 Uncertainty About Social Interactions Leads to the Evolution of Social Heuristics. *Nature Communications* **9**, 2151. (doi:10.1038/s41467-018-04493-1).

[73] Cheney, D.L. 2011 Extent and Limits of Cooperation in Animals. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 10902-10909. (doi:10.1073/pnas.1100291108).

[74] Dugatkin, L.A. 2002 Cooperation in Animals: An Evolutionary Overview. *Biology & Philosophy* **17**, 459-476.

[75] Milinski, M. & Wedekind, C. 1998 Working Memory Constrains Human Cooperation in the Prisoner's Dilemma. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 13755-13758.

[76] Stevens, J.R., Cushman, F.A. & Hauser, M.D. 2005 Evolving the Psychological Mechanisms for Cooperation. *Annual Review of Ecology, Evolution, and Systematics* **36**, 499-518. (doi:10.1146/annurev.ecolsys.36.113004.083814).

644
645

646 **Figure legend**

647

648 **Figure 1.** (A) Predator inspection arena with illustrative examples of movement of fish in
649 inspection lanes (red lines) (B) When focal fish had experienced defection by a shoal during
650 predator inspection they differed from other groups in a social partner choice paradigm. They
651 showed a preference for novel social partners over social partners that were ostensibly from
652 the shoal they had experienced while inspecting a predator (control = condition with no
653 predator present; **=significant at α=0.0125; error bars=±1 SEM).

654

655

Table 1. Results of the analysis of the main experiment testing for an effect of the inspection condition that fish were in (no predator present, i.e. control, versus predator present, i.e. experimental), the social environment that fish experienced during the inspection portion of a trial (cooperative vs. non-cooperative), the type of diet (daphnia or bloodworm) that novel shoaling partners had been fed on and their interactions. The significant interaction between inspection condition and social experience was further explored (Table 2). The significant effect of diet type was driven by an overall preference for fish that had been fed on a bloodworm diet.

Source	*F(1,121)*	*p*
Inspection condition	0.294	0.589
Social experience	5.491	**0.021**
Diet type	4.549	**0.035**
Inspection condition * Social experience	6.134	**0.015**
Inspection condition * Diet type	0.000	0.984
Social experience * Diet type	2.840	0.095
Inspection condition * Social experience * Diet type	0.062	0.804

667 **Table 2.** Results of the *post hoc* t-tests of significant interaction terms in the behavioural
668 dataset (see Table 1). Significance after Bonferroni-correction (α=0.0125) is shown in bold
669 and indicates a preference in the shoal-choice experiment for a novel social environment after
670 individuals have experienced defection.

Inspection condition	Social experience	t	df	p
No predator present	Cooperation	0.377	29	0.709
	Defection	0.353	32	0.726
Predator present	Cooperation	-1.675	32	0.104
	Defection	2.933	32	**0.006**

671

672

673 **Supplementary material for:**

674 **Trinidadian guppies use a social heuristic that can support cooperation among non-kin**

675 Safi K. Darden[1*], Richard James[2], James M. Cave[2], Josefine Bohr Brask[1] and Darren P. Croft[1]

676 [1]Centre for Research in Animal Behaviour, Department of Psychology, College of Life and
677 Environmental Sciences, University of Exeter, UK
678 [2]Department of Physics and Centre for Networks and Collective Behaviour, University of
679 Bath, UK
680

681 **Section 1: Evidencing that a 'Walk Away' social heuristic can lead to assortment of**
682 **cooperative agents in a population with fission-fusion group dynamics**

683 Social structures based around dynamic group membership are ubiquitous within the animal
684 kingdom [1, 2]. Since previous models investigating the extent to which a 'Walk Away' rule
685 can drive positive assortment by propensity to cooperate have not captured these social
686 dynamics [3-5, but see 6 where individuals can leave a group prior to any interaction], it is
687 unclear if a 'Walk Away' rule can generate such assortment in systems where groups
688 stochastically split and merge (i.e. the dynamic fission and fusion of groups typifying many
689 social species). We therefore use a simulation model to explore the proposition that a 'Walk
690 Away' heuristic can generate positive assortment of social interactions by individual
691 cooperative phenotypes in the highly dynamic social environments that typify many social
692 vertebrates.
693
694 We implemented an agent-based, steady-state stochastic simulation model of fission and fusion
695 in the spirit of existing merge and split models [7, 8] to generate conditions representing a
696 highly dynamic fission-fusion system (see detailed methods below). Our key addition was that
697 the phenotypes of the group members (45 obligate co-operators and 45 obligate defectors, each
698 with a given tolerance for defection, E_i) played a part in determining the membership of
699 daughter groups after fission. Briefly, in our model, we associated each fission event of a parent
700 group with a public-goods game, yielding a return R for each group member. An individual's
701 satisfaction at the outcome of the game was $S_i=R-E_{ii}$, where E_i is the individual's 'tolerance'
702 for defection (see detailed methods below). Satisfied agents ($S_i \geq 0$) joined either of two
703 daughter groups with equal probability. Dissatisfied agents ($S_i < 0$) could 'Walk Away', either
704 by forming a new group of their own, or by joining any one of the other groups in the
705 population, including the two daughter groups (Fig. S1A,B). From the simulation we collected
706 2500 independent censuses (every 10,000 timesteps) of group membership to form a weighted
707 network of associations (see detailed methods below). As a control, we ran a neutral model
708 where we randomised the membership of the groups recorded in each census in the 'Walk
709 Away' model.
710

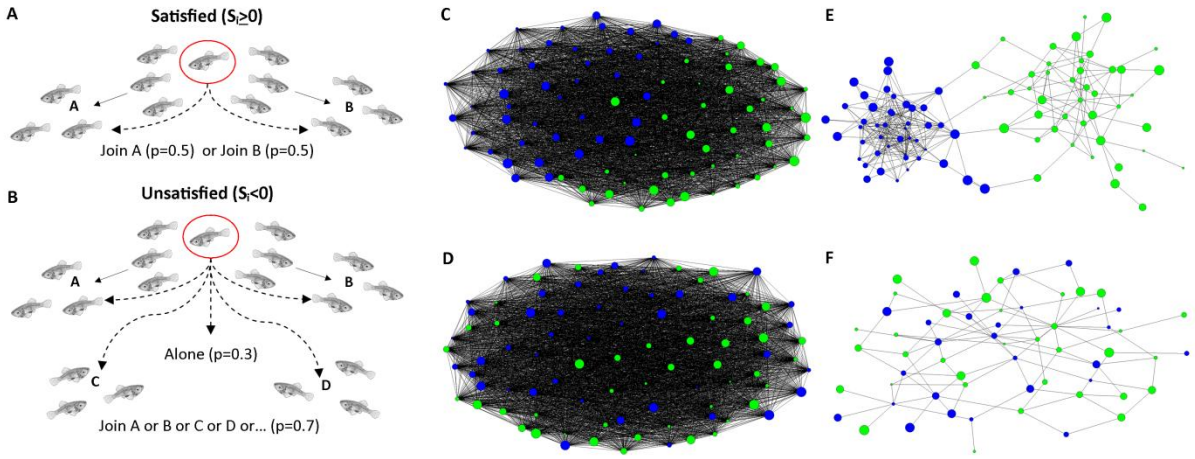

Fig S1. Simulation model 'Walk Away' rule implementation with illustrative graphical output. (A-B) Individual conditional movement decisions made at group fission when a 'Walk Away' rule is imposed on an agent-based, steady-state simulation model with fission–fusion dynamics. (A) 'Satisfied' individuals are those whose minimum return from being in the group is met ($R\text{-}E_i>0$, see text), while (B) 'unsatisfied' individuals are those whose minimum have not been met ($R\text{-}E_i<0$). (C-F) Graphs of interactions between agents in the model whose association indices are greater than (C-D) 0.042 and (E-F) 0.06 with (C,E) a 'Walk Away' rule imposed and (D,F) a neutral model. Node colour indicates phenotype (green=co-operator, blue=defector), node size indicates, E, as higher (smaller nodes due to lower E) and lower (larger nodes due to higher E) tolerance for defection (range 0.2-0.8), lines indicate dyadic connections greater than the respective filtering thresholds.

The results of the model demonstrate that even against a dynamic background of fission and fusion, a simple 'Walk Away' rule can drive social assortment by cooperative phenotype (Fig. S1C,E and Fig. S2); when agents use a walk away strategy, the assortment of social ties by cooperative phenotype within the population become significantly greater than zero with increasing tie strength, which is not the case in a neutral model (Figs. S1D,F, S2A and S3).

Detailed methods

Agent-based simulation model

The model population consisted of 90 agents, 45 obligate co-operators and 45 obligate defectors. Agents were in groups, whose size and composition were subject to fission-fusion dynamics implemented through probabilistic rules. At each timestep there was a small probability ($3.5\text{x}10^{-5}\sqrt{(s_1 s_2)}$) that two groups of size s_1 and s_2 would fuse to form a group of size s_1+s_2; thus large groups were more likely to fuse than were small groups. There was also a small constant probability (0.004, irrespective of size) that a given group would split and decision rules were implemented at these fission events. Our split and merge rules allowed us to mimic a biologically realistic monotonically decreasing group size as typically observed in fission fusion social systems [9].

Each agent was assigned a phenotype along a gradient of values, spread evenly in the range 0.2 to 0.8, that determined its "expectation", E_i, of the cooperative behaviour of others in the group. For example, the lowest E-values (0.2 to 0.4) had lower expectations and therefore can be

considered more 'tolerant' of defection. At the moment of fission we assumed that the focal group had just undergone a cooperative game. Each co-operator in the group contributed 1 point to a shared "pool", defectors contributed 0. The value of the pool was multiplied by 1.9 [as in 3, 4], then shared equally among all group members. This "return" from the game, R, minus an agent's expectation E_i, determined its 'satisfaction' with being in the group at the time of the fission event: $S_i=R-E_i$. The satisfied agents (those with $S_i \geq 0$) split into two daughter groups (Fig. S1). Each satisfied agent had a 50% chance of being placed in each of the two groups. Agents that were not satisfied ($S_i<0$) had a tendency to 'walk away'; they either formed a group of N=1 or joined an existing group with equal likelihood of joining any particular group, including each of the daughter groups formed by the fission of satisfied agents (Fig. S1). After 50,000 timesteps at which point the model had reached steady-state (dynamic equilibrium), we monitored group membership every 10,000 timesteps, in a series of 2,500 censuses of the population. The 10,000 timestep interval was derived from our expectation in the neutral model that every agent had had the opportunity to be in a group with every other agent over that period, which allowed us to produce censuses free of sequential correlation. For these associations we constructed a weighted 90x90 association

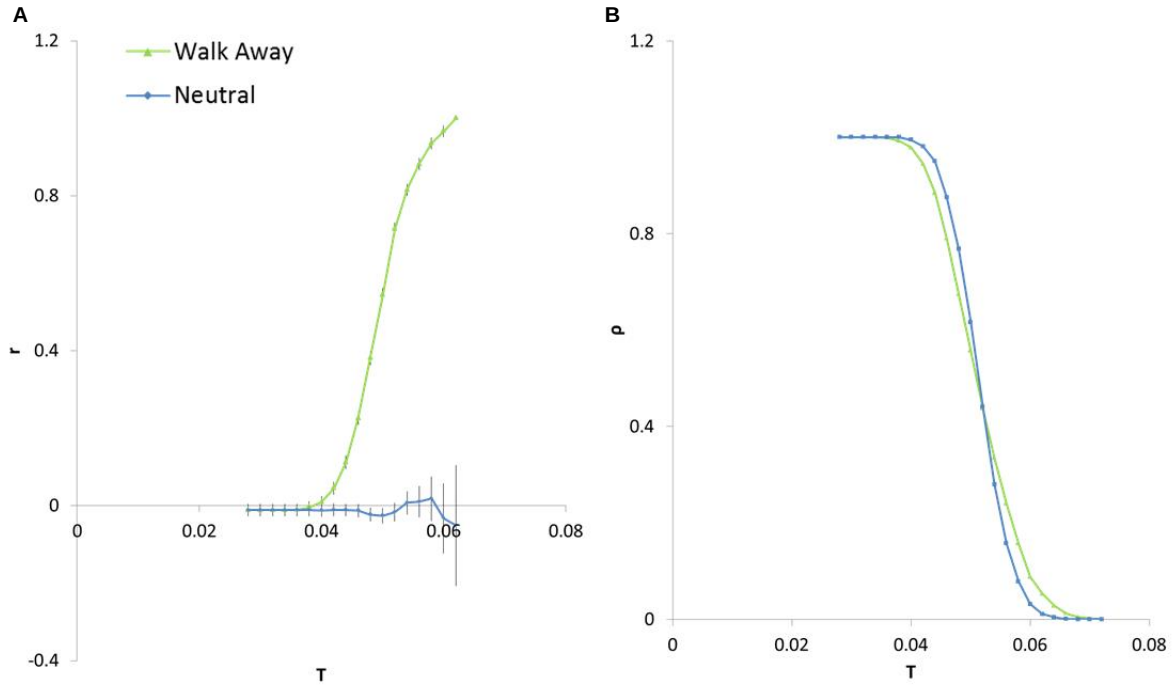

Fig S2. Assortment by cooperativeness in the social networks sampled from an agent-based, steady-state simulation model with fission–fusion dynamics. (A) The assortivity coefficient, r, is an indicator of the overall assortivity of associations in the population by cooperative phenotype (see Methods) with a 'Walk Away' rule imposed (green) and without such a rule (blue). T is the threshold over which agents must associate to be assigned a tie strength of one in a binary association matrix. Error bars = +/- 1σ and indicate whether the value of r differs from zero at a given T (see Methods). (B) The fraction of ties, ρ, that have an association index greater than our filtering threshold, T, in our 'Walk Away' and neutral models. The decrease reflects the fact that a smaller fraction of the population had stronger ties.

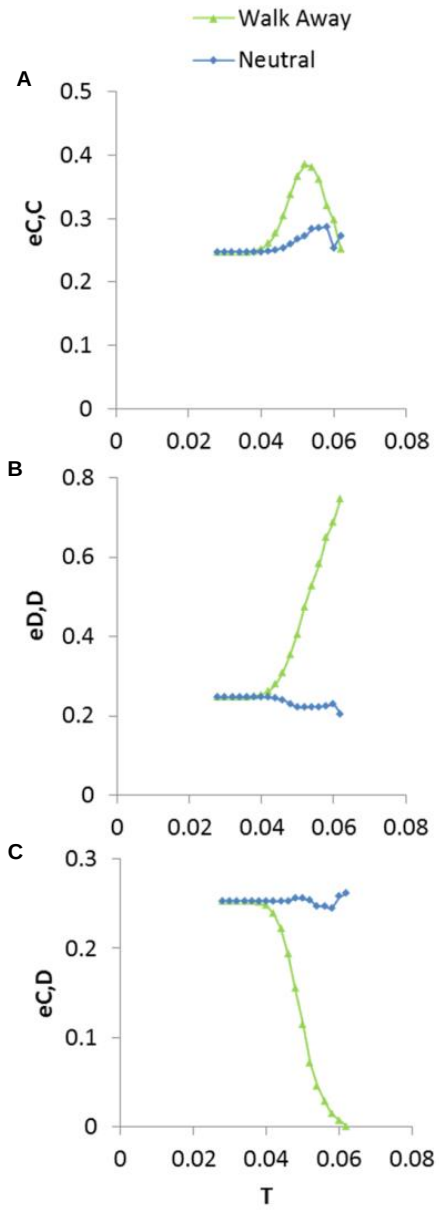

Fig. S3. Frequency of tie 'types' in the sampled networks. (A-C) The proportion of edges in the network, e, that are represented by the three phenotypic dyad types (C,C = co-operator-co-operator, C,D = co-operator-defector, D,D = defector-defector) with the 'Walk Away' rule implemented (green) and in the null model (blue).

matrix W, whose entry W_{ij} was the fraction of censuses in which agents i and j were in the same group. All agents occurred at least once with all others, so all $W_{ij}>0$. Our neutral model used the same group sizes as the original model at every census, but the groups were populated randomly with respect to S.

Analysis of simulation data

To analyse whether the implementation of a 'Walk Away' rule was sufficient to maintain long-term assortment in our population, despite rapid fission-fusion dynamics, we constructed a

series of binary matrices A(T) whose entry $A_{ij}(T)$ was 1 if $W_{ij} \geq T$, and 0 otherwise. T is a threshold fraction of times agents were found in the same group in our 2,500 censuses. As T increased, the density of A (ρ , the fraction of elements that are 1) decreased reflecting the fact strong associations were found between a smaller fraction of agents (Fig. S1). For each A(T), we computed Newman's assortativity coefficient r [10] which measures whether there are more CC and/or DD pairs in our groups than if edges were wired at random (Fig. S2). This is our measure of assortment in the population. A jack-knife procedure was used to test whether the computed values of r were significantly greater than zero in each of our models [10].

References

[1] Aureli F, Schaffner CM, Boesch C, Bearder SK, Call J, Chapman CA, Connor R, di Fiore A, Dunbar RIM, Henzi SP, et al. 2008 Fission-Fusion Dynamics: New Research Frameworks. *Current Anthropology* **49**, 627-654. (doi:10.1086/586708).

[2] Couzin ID. 2006 Behavioral Ecology: Social Organization in Fission–Fusion Societies. *Current Biology* **16**, R169-R171. (doi:https://doi.org/10.1016/j.cub.2006.02.042).

[3] Aktipis CA. 2004 Know When to Walk Away: Contingent Movement and the Evolution of Cooperation. *Journal of Theoretical Biology* **231**, 249-260.

[4] Aktipis CA. 2011 Is Cooperation Viable in Mobile Organisms? Simple Walk Away Rule Favors the Evolution of Cooperation in Groups. *Evolution and Human Behavior* **32**, 263-276. (doi:http://dx.doi.org/10.1016/j.evolhumbehav.2011.01.002).

[5] Schuessler R. 1989 Exit Threats and Cooperation under Anonymity. *The Journal of Conflict Resolution* **33**, 728-749.

[6] Hamilton IM & Taborsky M 2005 Contingent Movement and Cooperation Evolve under Generalized Reciprocity. *Proceedings of the Royal Society B: Biological Sciences* **272**, 2259.

[7] Niwa, H-S. 2003 Power-Law Versus Exponential Distributions of Animal Group Sizes. *Journal of Theoretical Biology* **224**, 451-457. (doi:http://dx.doi.org/10.1016/S0022-5193(03)00192-9).

[8] Gueron S Levin SA. 1995 The Dynamics of Group Formation. *Mathematical Biosciences* **128**, 243-264. (doi:http://dx.doi.org/10.1016/0025-5564(94)00074-A).

[9] Okubo A. 1986 Dynamical Aspects of Animal Grouping: Swarms, Schools, Flocks, and Herds. *Advances in Biophysics* **22**, 1-94. (doi:https://doi.org/10.1016/0065-227X(86)90003-1).

[10] Newman MEJ. 2003 Mixing Patterns in Networks. *Physical Review E* **67**, art. no.-026126.

Section 2: Supplementary detail on methods and results of the main experiment.

Table S1. Number of focal individuals tested at each level in the study's main experiment. N denotes the number of biological replicates (focal individuals).

Inspection condition	Social experience	Diet type (inspection phase)	N
No predator present	Cooperation	Bloodworm	14
		Daphnia sp.	16
		Total	**30**
	Defection	Bloodworm	16
		Daphnia sp.	17
		Total	**33**
	Total	Bloodworm	30
		Daphnia sp.	33
		Total	**63**
Predator present	Cooperation	Bloodworm	16
		Daphnia sp.	17
		Total	**33**
	Defection	Bloodworm	16
		Daphnia sp.	17
		Total	**33**
	Total	Bloodworm	32
		Daphnia sp.	34
		Total	**66**
Total	Cooperation	Bloodworm	30
		Daphnia sp.	33
		Total	**63**
	Defection	Bloodworm	32
		Daphnia sp.	34
		Total	**66**
	Total	Bloodworm	62
		Daphnia sp.	67
		Total	**129**

Table S2. Results of the analysis of the main experiment testing for an effect of the inspection condition that fish were in (no predator present, i.e. control, versus predator present, i.e. experimental), the social environment that fish experienced during the inspection portion of a trial (cooperative vs. non-cooperative), the type of diet (daphnia or bloodworm) that novel shoaling partners had been fed on and their interactions including the inspection behaviour of focal individuals in the model (removed in final model). *Note:* we did not have inspection data for 4 focal individuals in the control inspection condition (no predator present) due to video failures.

Source	*F(1,116)*	*p*
Inspection behaviour	0.393	0.532
Inspection condition	0.749	0.388
Social experience	5.915	**0.017**
Diet type	5.171	**0.025**
Inspection condition * Social experience	5.714	**0.018**
Inspection condition * Diet type	0.015	0.903
Social experience * Diet type	2.517	0.115
Inspection condition * Social experience * Diet type	0.116	0.734